Reversal of Acute and Chronic Synovial Inflammation by Anti-Transforming Growth Factor β

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Summary

Transforming growth factor β (TGF-β) induces leukocyte recruitment and activation, events central to an inflammatory response. In this study, we demonstrate that antagonism of TGF-β with a neutralizing antibody not only blocks inflammatory cell accumulation, but also tissue pathology in an experimental model of chronic erosive polyarthritis. Intraarticular injection of monoclonal antibody 1D11.16, which inhibits both TGF-β1 and TGF-β2 bioactivity, into animals receiving an arthropathic dose of bacterial cell walls significantly inhibits arthritis. Inhibition was observed with a single injection of 50 μg antibody, and a 1-mg injection blocked acute inflammation >75% compared with the contralateral joints injected with an irrelevant isotype control antibody (MOPC21) as quantitated by an articular index (AI = 0.93 ± 0.23 for 1D11.16, and AI = 4.0 ± 0 on day 4; p <0.001). Moreover, suppression of the acute arthritis achieved with a single injection of antibody was sustained into the chronic, destructive phase of the disease (on day 18, AI = 0.93 ± 0.07 vs. AI = 2.6 ± 0.5; p <0.01). The decreased inflammatory index associated with anti-TGF-β treatment was consistent with histopathologic and radiologic evidence of a therapeutic response. These data implicate TGF-β as a profound agonist not only in the early events responsible for synovial inflammation, but also in the chronicity of streptococcal cell wall fragment–induced inflammation culminating in destructive pathology. Interrupting the cycle of leukocyte recruitment and activation with TGF-β antagonists may provide a mechanism for resolution of chronic destructive lesions.

Transforming growth factor-β (TGF-β) has emerged as an unrivaled natural inhibitor of many immune-mediated pathways. Not only does TGF-β inhibit a variety of immune functions in vitro (reviewed in reference 1), but more recent evidence has documented the ability of this cytokine to reverse inflammation and immune-mediated pathology in vivo (1–5). However, therapeutic efficacy has been reported only after systemic administration of TGF-β, whereas localized injections of TGF-β appear to precipitate a self-limiting inflammatory response with accumulation of leukocytes (6–8). These data are consistent with in vitro evidence that TGF-β is a strong chemoattractant for neutrophils, monocytes, and T cells (9–11). Moreover, TGF-β facilitates the migration of monocytes into tissue by augmenting integrin expression and production of matrix-degrading enzymes (1). Additionally, TGF-β induces FcγRIII expression on blood monocytes (12) and triggers the production of IL-1, TNF, IL-6, and TGF-β itself (13, 14), thereby expanding the inflammatory network. These observations, along with numerous demonstrations of active TGF-β peptide within inflammatory lesions (1), implicate TGF-β as an early agonist of inflammatory events. However, within a particular inflammatory site, it may be unclear whether TGF-β is functioning as a mediator of inflammation or in the suppression of these events.

One approach to this question, not yet fully explored, is the use of TGF-β antagonists to establish cause and effect of this potent cytokine. In this study, we have utilized a neutralizing antibody to TGF-β and administered it locally within an inflammatory site in the synovium of animals receiving an arthropathic dose of bacterial cell walls. The group A streptococcal cell wall fragments (SCW) induce a characteristic pattern of acute synovitis followed by chronic mononuclear cell–mediated destructive lesions (2). The antibody to TGF-β, as compared with an irrelevant antibody, clearly suppressed leukocyte accumulation and the sequelae of events culminating in joint destruction. Thus, TGF-β, released early in the inflammatory cascade and concentrated locally, appears to be a protagonist for those inflammatory events leading to synovitis and T cell–dependent erosive joint disease.

Materials and Methods

Induction and Treatment of Arthritis. Arthritis was induced in pathogen-free female LEW rats (Harlan Sprague Dawley, Indi-

EXHIBIT 4

anapolis, IN) (~100 g) by intraperitoneal injection of cell wall fragments from group A streptococci (SCW) (30 μg rhamnose/g body weight) (2). The course of acute and chronic joint pathology was clinically monitored by determining the articular index (AI), which reflects the degree of joint erythema, swelling, and distortion on a scale of 0 (normal) to 4 (severe inflammation) for each joint. Radiographs taken with direct exposure (1:1) on X-Omat TL Kodak film using 60-kV, 345-mA, 60-s exposure were evaluated for soft tissue swelling, joint space narrowing, bone erosions, and deformity.

SCW-injected and control LEW rats were injected intraarticularly into one of the hind ankles with neutralizing mAb to TGF-β (1D11.16) (15) in 25 μl PBS, PBS only, or an irrelevant isotype control mouse myeloma Ig (MOPC21, IgG1) at the indicated concentrations and times relative to SCW injection. Antibodies 1D11.16 and MOPC21 contained <0.8 ng/mg endotoxin as determined by the limulus assay.

Histopathology. At various intervals, tissue specimens were fixed in 10% buffered formalin, decalcified in 10% EDTA, sectioned, and stained with hematoxylin and eosin for histopathologic analysis as described (2).

RNA Isolation and Northern Analysis. Total RNA was isolated from excised synovial tissues (6), and 5 μg total RNA was fractionated on 1% gels before being transferred to nitrocellulose filters. The blots were prehybridized for 4 h and then hybridized overnight with ^{32}P-labeled cDNA probe for human IL-1β, and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as described (6). The blots were then exposed to phosphor screens and analyzed using a Phosphor-Imager (Molecular Dynamics, Sunnyvale, CA). The resultant images were then printed on a Laser Jet III (Hewlett-Packard Co., Palo Alto, CA) grey-scale printer and quantitated using ImageQuant (Molecular Dynamics) software.

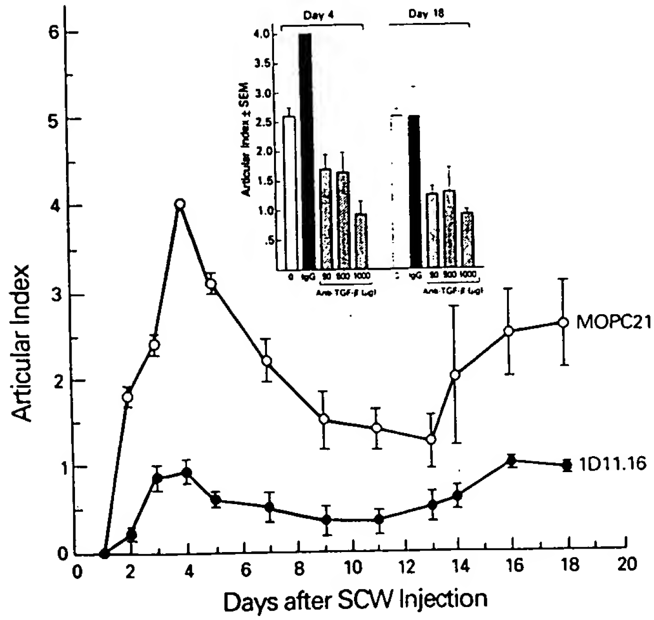

Figure 1. Inhibition of acute and chronic arthritis by anti-TGF-β. Animals were injected in one hind joint with 1 mg mAb 1D11.16 or MOPC21 just before the intraperitoneal injection of an arthropathic dose of SCW on day 0. Articular indices (AI) were determined at the indicated intervals and data represent the mean ± SE for four animals. (*Inset*) Animals injected with SCW were treated with MOPC21 at 0.5 mg or with 0.05, 0.5, or 1.0 mg anti-TGF-β in 25 μl PBS on day 0, and AI were determined during the acute (day 4) and chronic phase (day 18). Data represent the mean ± SE of four animals for each dose of antibody.

Results

Clinically Evident Suppression of Arthritic Lesions by Anti-TGF-β. Dissemination and localization of SCW to the synovium initiates an acute inflammatory response clinically detected by an elevated articular index (AI) within hours and maximal by 3–5 d (Fig. 1). The anti-TGF-β mAb 1D11.16, which neutralizes both TGF-β1 and TGF-β2, was administered locally into the synovial space just before the systemic injection of the arthritogenic dose of SCW. In those joints receiving a single intraarticular injection of 1 mg anti-TGF-β, the AI was reduced ≥75% at 24 h compared with the contralateral joints injected with an equivalent amount of the isotype control antibody (Fig. 1). During the peak of the acute inflammatory responses (days 3–5), the AI for the animals receiving anti-TGF-β remained significantly below the clinical index for those injected with MOPC21 (AI = 0.93 ± 0.23 for 1D11.16 vs. 4.0 ± 0 for MOPC21; p <0.001). Neither 1D11.16 nor MOPC21 injected into naive joints of nonarthritic animals independently induced inflammation (AI = 0). Suppression of arthritis by anti-TGF-β was localized in that no suppression was seen in the contralateral hind limb or the two front limbs in the absence of direct injection of antibody.

After the nadir (days 7–12), in which the acute phase subsides and the cellular infiltrate within the synovial tissue becomes predominantly mononuclear leading to joint destruction, the AI of the control animals treated with MOPC21 typically increased, reaching maximal scores within 2–3 wk after the SCW injection (Fig. 1). In marked contrast, animals injected intraarticularly with but a single 1-mg dose of anti-TGF-β on day 0 exhibited clinical scores that remained significantly below the control levels for the duration of the experiment (day 18; p <0.005).

To determine if lower doses of the antibody to TGF-β could modulate the course of arthritis, a single injection of 0.05–1 mg 1D11.16 was administered coincident with the SCW. Whereas MOPC21 often exacerbated the synovitis, as shown in Fig. 1, *inset*, a significant suppression of both acute (day 4) and chronic (day 18) inflammation was apparent at concentrations as low as 50 μg of anti-TGF-β, with maximal benefit from the single 1-mg injection. Strikingly, the single injection of antibody suppressed not only the SCW-induced inflammation responsible for soft tissue swelling (Figs. 1 and 2 *A*), but also the subsequent events essential for the evolution of chronic arthritis, as evidenced by reduced destruction of bone and cartilage (Fig. 2 *B*). Moreover, when anti-TGF-β (0.5–1.0 mg, i.a.) was not administered until after the chronic phase of the response had begun (day 13), the antibody was still significantly inhibitory (AI = 3.2 ± .45 for MOPC21 vs. 1.0 ± 0 for 1D11.16, day 18; p <0.05).

Anti-TGF-β Suppresses Inflammatory Cell Recruitment, Synovitis, and Bone Resorption. SCW localization to the synovium induces a characteristic pattern of leukocyte infiltration (Fig.

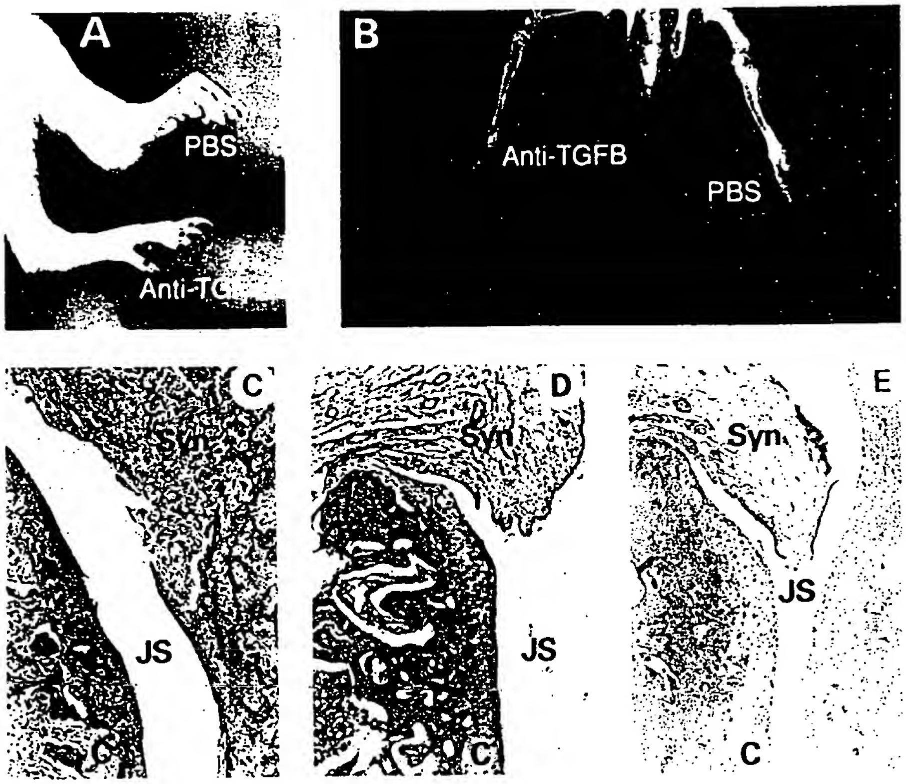

Figure 2. Anti-TGF-β inhibits joint swelling, bone deformities, and histopathology. Based on joint swelling (*A*) and radiologic evaluation (*B*) at 4 wk, arthritic joints receiving a single intraarticular injection of anti-TGF-β (0.7 mg) on day 0 exhibited reduced inflammation and tissue destruction as compared with the contralateral joint injected with PBS (shown) or MOPC 21. Animals receiving an arthropathic dose of SCW (*C* and *D*) or no SCW (*E*) were injected intraarticularly with TGF-β mAb (*D* and *E*) or MOPC21 (*C*). Tissues were excised and processed for histopathology after 4 wk.

2 *C*) that is markedly inhibited in the synovium of SCW-injected animals receiving anti-TGF-β (Fig. 2 *D*). Whether evaluated at 3 d (not shown) or 4 wk (Fig. 2) after SCW-induced arthritis, the synovial tissues of anti-TGF-β-treated animals exhibited a significant decrease in inflammatory cells, more closely resembling the control synovium (Fig. 2 *E*) than the arthritic synovium (Fig. 2 *C*).

Corresponding to the decrease in synovitis, a marked reduction in pannus, bone erosion, and cartilage degradation was evident. Whereas pronounced osteoclast formation and bone resorption were observed in arthritic joints with or without MOPC21 (Fig. 3 *A*), osteoclasts were seldom seen in 1D11.16-treated joint tissues (Fig. 3 *B*). In parallel with the histopathologic evidence of reduced tissue destruction, radiologic examination of antibody-treated and untreated joints revealed diminished soft tissue swelling and minimal bone abnormalities after anti-TGF-β administration (Fig. 2 *B*). Because TGF-β is not known to directly influence bone resorption, we addressed the effects of antagonizing TGF-β activity on IL-1 that does promote osteoclast activation. Synovial tissues from arthritic animals treated intraarticularly with MOPC21 (500 μg) or anti-TGF-β (0.05–1.0 mg) were excised, RNA isolated, and probed with IL-1β cDNA by Northern analysis. A dose-dependent decrease in IL-1β mRNA was detected in the synovia receiving anti-TGF-β as compared with the isotype control antibody (Fig. 3 *C*). These data implicate a causal role for TGF-β in leukocyte recruitment and activation leading to the release of cytokines essential for SCW-induced joint pathology.

Discussion

TGF-β has been identified in rheumatoid synovium (12, 16, 17), local administration of TGF-β to arthritic synovium in experimental models exacerbates the pathology (18), and intraarticular injections of the cytokine independently initiate a self-limiting synovitis (6, 7), all pointing to a protagonist role for TGF-β in the evolution of arthritis. As more direct evidence, we now demonstrate that neutralizing antibodies to TGF-β markedly suppress both the acute and chronic phases of arthritis induced by SCW. How the antibody exerts this antiinflammatory effect is uncertain, but the most striking consequence is the paucity of leukocytes in the synovium. Anti-TGF-β most likely has a primary effect in blocking leukocyte recruitment into the synovium after deposition of the SCW by neutralizing the potent chemotactic activity of this cytokine and its ability to augment adhesion. TGF-β is released after exposure of mononuclear phagocytes to SCW (19), and is likely present early after localization of the SCW fragments in the synovium. Not only is TGF-β chemotactic (9–11), but it increases monocyte expression of $\alpha_3\beta_1$ and $\alpha_5\beta_1$ integrins, which can promote cell matrix interactions and localization of these cells at sites of inflammation (20). Through its influence on monocyte type IV collagenase production, TGF-β also may contribute to the extravascularization of these cells (20). By antagonizing these early activities of TGF-β, the requisite cellular influx appears to be inhibited, compromising the entire inflammatory response.

In addition to its recruitment potential, TGF-β elicits other activities of undifferentiated monocytes, including CD16 ex-

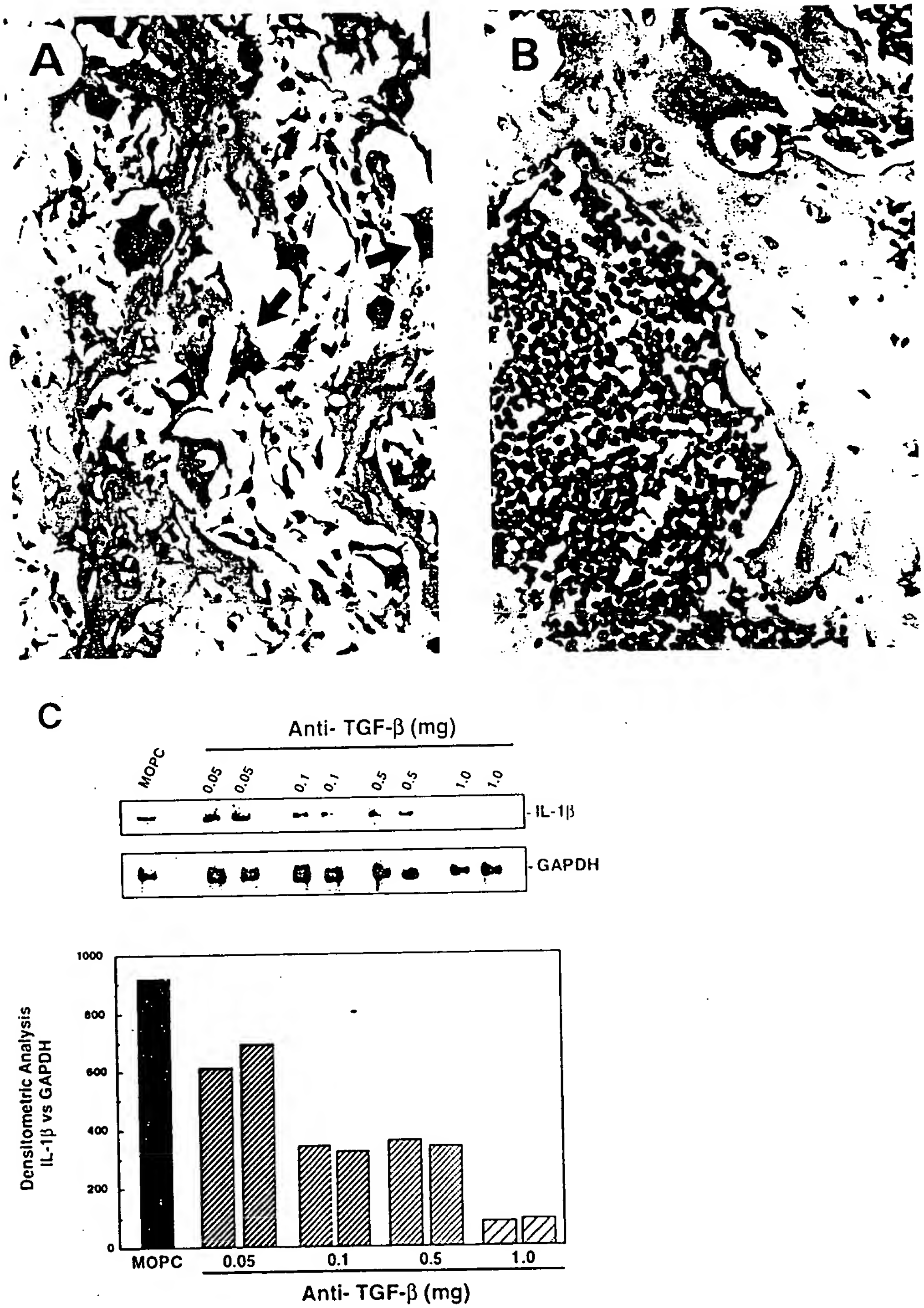

Figure 3. Anti-TGF-β inhibits osteoclasts, bone resorption, and IL-1. SCW-induced arthritic animals were injected intraarticularly with MOPC21 (*A*) or anti-TGF-β (*B*) on day 0, and joint tissues excised 4 wk later were processed for histopathologic analysis. (*C*) Synovial tissues were excised from arthritic animals injected 18 d previously with MOPC21 (0.5 mg) or with increasing concentrations of anti-TGF-β (0.05–1.0 mg). Total RNA was extracted, and 5.0-μg aliquots were subjected to Northern analysis using IL-1β and GAPDH cDNA probes. Blots were scanned, and the histogram represents the densitometric ratio of the IL-1β to GAPDH signal. Data are representative of two experiments.

pression (12) and the induction of TNF-α, IL-1, and IL-6 (9, 13, 14), which are important in the cytokine cascade responsible for development of an immunologic challenge. Based on recent evidence that TGF-β promotes proliferation and development of CD4$^+$ T cell precursors into Th cell populations (Th1) (21, 22), it is not surprising that inhibition of TGF-β activity diminishes the SCW antigen–dependent immune events. Besides the effects on lymphocyte and monocyte phenotype and function, a dramatic reduction in osteoclasts was apparent in the antibody-treated joints, but not in the joints receiving MOPC21. Since TGF-β is thought to stimulate osteoblast growth and bone formation (23), and to block osteoclast activity (24), this inhibitory effect of anti-TGF-β may be indirect, the consequence of impairing the antecedent inflammation and immune response.

Although TGF-β identified within inflammatory lesions

(1, 12, 16, 17) has generally been considered to be functioning in a suppressive capacity in immune and inflammatory events, one must now also consider that TGF-β is, in fact, contributing to the evolution of these events. Antagonism of TGF-β activity locally interrupts, rather than enhances, the T cell–dependent immune response in the SCW-induced arthritis model.

In inflammation and repair, the role of TGF-β may focus initially on recruitment and activation of immature leukocyte populations, whereas TGF-β may suppress inflammatory functions in these cells after they become activated. For example, TGF-β has disparate effects on immature monocytes and on differentiated macrophages. Whereas blood monocytes are exquisitely sensitive to recruitment and activation by TGF-β, once these cells undergo differentiation they experience TGF-β receptor downregulation with loss of sensitivity to TGF-β stimulation (25), and inactivation by this peptide (26). After subserving their functions to process and eliminate foreign agents or pathogens, mononuclear phagocyte responsiveness to TGF-β is reversed during cell maturation. Similarly, immature T cells are susceptible to upregulation by TGF-β (21, 22), whereas once activated, T cells express increased TGF-β receptors and are growth arrested after ligand binding (27). Thus, TGF-β is a pivotal mediator, beneficial both in the initiation and in the resolution of fundamental inflammatory processes (1). Importantly, as leukocyte sensitivity to TGF-β stimulation wanes, the influence of this peptide on events central to tissue repair becomes apparent as healing occurs (1, 8, 28). However, in chronic lesions, excess production or lack of regulation of this otherwise normal growth factor may lead to excessive leukocyte recruitment and activation with the potential for tissue destruction and/or fibrosis. In circumstances where TGF-β contributes to pathogenesis, TGF-β antagonists may be useful in breaking the cycle of cell recruitment and activation.

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